Genotypic Diversity of the Wheat leaf blotch pathogen (*Septoria tritici*) in Buenos Aires Province

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Summary: The population structure and genotypic diversity of *Septoria tritici* Rob ex Desm. from two crop field populations in Buenos Aires Province were studied with DNA restriction fragment length polymorphism. Hierarchical samples were taken from different cultivars. A total of 120 single spore isolates was subject to molecular analysis to compare the genetic structure of Los Hornos and Balcarce populations separated by 500km. Eight RFLP loci and 1DNA fingerprinting were used. Among the total of the isolates, 24 RFLP alleles were detected from the first population and 22 from the second. Gene and genotype diversity were high in both populations. Different haplotypes for each region were identified. Identical haplotypes were clustered in the same location in the field. The mean gene diversity and the mean genotypic diversity were high for the 8 loci of RFLP. This means that Balcarce and Los Hornos were significantly different. The X^2 was significant (P>0.005) only for two RFLP locus, then both populations were not independant. They had to be compared with an European and an American populations. Balcarce population was very different to all of them. Both Argentine populations were very similar at regional level but strongly different at a continental level. The greatest genetic variability between continents belonged to Argentine population. The amount of gene flow was high when all the populations were compared.

Key words: DNA hybridization, *Septoria tritici, Mycosphaerella graminicola*, RFLP, epidemiology, genetic diversity, genotypic diversity.

Resumen: Diversidad genotípica del patógeno de la mancha de la hoja del trigo (*Septoria tritici*) en la provincia de Buenos Aires. Se estudió la estructura poblacional y la diversidad genética y genotípica de dos poblaciones de *Septoria tritici* Rob ex Desm. con la técnica de RFLP Un total de 120 aislamientos monospóricos colectada en campos experimentales de la Provincia de Buenos Aires se analizaron molecularmente, comparando la estructura genética de dos poblaciones separadas por 500 km. Se experimentó con 8 loci de RFLP y una sonda de ADN fingerprinting. Sobre la totalidad de los aislamientos se detectaron 24 alelos de RFLP para la localidad de Los Hornos y 22 para Balcarce. En la población de Los Hornos entre 58 aislamientos se reconocieron 35 haplotipos multilocus y en Balcarce, entre 62 aislamientos, 39 haplotipos, significando que ambas poblaciones son diferentes. Las diversidades genética y genotípica fueron altas en ambas poblaciones. La diferencia entre poblaciones, para las frecuencias de alelos, se calculó con el test de contingencia X^2; como estas fueron solo significativas para dos locus de RFLP (P>0.005) se procedió a comparar las poblaciones locales con una de europea y otra americana. Las poblaciones en Argentina fueron muy distintas a las otras poblaciones analizadas. Se interpretó que las poblaciones argentinas fueron similares a nivel regional pero difirieron significativamente de las de Suiza y Oregón, para los loci analizados.

Palabras clave: hibridización de ADN, *Septoria tritici, Mycosphaerella graminicola*, RFLP, epidemiología, diversidad genética ,diversidad genotípica.

Introduction

Foliar fungal pathogens possess significant threats to grain crop production. *Septoria* diseases of wheat (*Mycosphaerella graminicola*, anamorph *Septoria tritici)* cause economically significant yield losses in most of the wheat growing areas of the world. Losses can range from 31 to 54% in climates conductive to diseases development (Eyal *et al.,* 1985). In Argentina, Annone *et al.* (1991, 1993) reported yield losses from 20 to 50% and Simón *et al.* (1996) found reductions in thousand kernel weights of 3 to 13%.

The primary genetic marker used for *S. tritici* in Argentina, was virulence (Cordo & Arriaga, 1990; Cordo *et al.,* 1990/91; Giecco *et al.,* 2004; Perelló *et al.,* 1990). It was studied on a set of wheat differentials that varied in their level of resistance. However, virulence in this fungus is a character that is very

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sensitive to environmental conditions, originating difficulties to evaluate it in a reproducible manner

The population genetic structure of *S. tritici* has been characterized extensively by combining computer modeling (Zhan *et al.,* 2004) experimental evolution approaches (Zhan *et al.,* 1998, 2002) and population surveys based on restriction fragment length polymorphism (RFLP) (Zhan *et al.,* 2001, 2003, 2004). Populations in this fungus are in genetic equilibrium as well as in drift migration equilibrium (Chen *et al.,* 1996) attributed to a high rate of sexual recombination. Field populations sampled from different regions and continents shared similar RFLP frequencies suggesting that substantial gene flow has occurred across long distances (Zhan *et al.,* 2003).

Genetic variations using restriction fragment length polymorphism (RFLP) began to be studied recently (Cordo *et al.* 1998, Cordo *et al.* 2006) in Argentina. Moreover the major wheat-growing regions of Buenos Aires Province have not been sampled extensively and it is not known whether results from other parts of the world are representative of these wheat growing regions. A set of genetic markers based on RFLP that could be used to estimate the amount and distribution of genetic variability, gene and genotype diversity, gene flow and the DNA fingerprints to identify clones. (Boerger *et al.,* 1993; McDonald & Martínez, 1990a, 1990b, 1991; McDonald *et al.,* 1995; Shaw & Royle, 1989) were developed in USA and Europe based on DNA radioactive labeling of the probe (McDonald & Martínez, 1990a).

The objectives of this work was to apply RFLP markers to compare the genetic structure of the Los Hornos and Balcarce populations that are 500 km distant from each other and to assess the potential for gene flow between both populations.

Materials and Methods

The leaf infected tissues were collected from plants on GS10.1 stage (Zadocks *et al.*, 1974) in Los Hornos and Balcarce (Buenos Aires Province, Argentina) (Fig. 1). The hierarchical sampling method was used with each plant to collect *S. tritici* isolates from a naturally infected wheat field of Los Hornos. Fifty eight isolates originated from 39 lesions on 13 leaves were sampled at three different times in a single field. Each of them was approximately 10 m^2 in area (Table 1).

A different strategy was used to sample a population of the Balcarce location. Collection was made from some experimental cultivars in the Agricultural Experimental Station, INTA. Cultivars that differed in resistance to *S.tritici* were planted in repetitions of 6 rows per cultivar. Thirty seven infected leaves in total, were randomly chosen from different cultivars. Sixty two isolates originated from 57 lesions were sampled. The total sampled area was 80 m^2 (Table 2). The infected wheat leaves were air-dried at room temperature for 2 wk before the fungi isolations were done. Only one single-spore isolate was obtained with the disinfections technique (alcohol 70% and Cl$_2$ Hg 1/1000 g/ml) and cultivated on PDA (2%) (Plant Pathologist's Pocketbook, 1968). The isolates were grown in yeast sucrose broth at room temperature (18-22 Cº) during 10 days with shaking (150 rpm) to obtain the spore concentration for further DNA extraction (McDonald & Martínez, 1990)

DNA was extracted from each isolate by a CTAB extraction protocol described previously (McDonald & Martínez, 1990; Cordo *et al.,* 2006).

Anonymous probes for this study were selected using three criteria: 1) one repetitive probe to detect fingerprinting (pSTL70), 2) probes that produced a strong hybridization signal and hybridized to only one or two fragments (pSTL10, pSTL31, pSTS43, pSTS14) and 3) probes that hybridized to fragments between 0.5-6.0kb to insure adequate resolution of all restriction fragments present in our sample (pSTL53, pSTS192A, pSTS192B, pSTS2). All probes were handed over by Dr. McDonald. Purified DNA (5ug.) was digested individually with the PstI restriction enzyme. DNA fragments were separated on 0.75% agarose gels and then transferred to a nylon membrane by the alkaline transfer method (Reed & Man, 1985) as recommended by the manufacturer (BioRad, Hercules C.A.).

The plasmid was recovered from the *Escherichia coli* (HB101 strain) culture with the wizard Maxiprep DNA Purification System (Promega). Probes were labeled by radioactivity with P32, by nick translation, following the manufacturer's recommendations (BRL, Gaithersburg, MD). The single locus probes used for this experiment are shown in Table 4.

Each probe-enzyme combination defined an RFLP locus. DNA fragments or combinations of fragments of different sizes were treated as alleles at each RFLP locus. The number of isolates used in each analysis varied because of differences in the sampling methods for the two populations. Sample sizes of each locus also varied because data from some isolates were incomplete as a result of partial digestion, differences in the amount of DNA loaded in each line, and
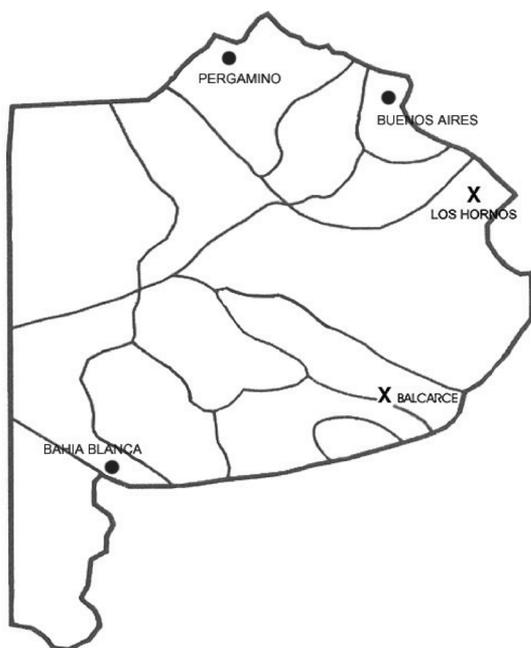
Fig. 1. Wheat productive areas in Buenos Aires province. **X** is indicating the place of sampling.

occasional nonspecific background hybridization. Only alleles that could be scored unambiguously were included in each analysis.

Genetic variation in each field population was quantified using measures of gene diversity (Nei, 1972). This measure of population differentiation was calculated to examine inter-population diversity among field populations. In addition a measure of

genotypic diversity based on the number of multilocus haplotypes was calculated in each population. The total gene diversity was partitioned into several spatial components using hierarchical gene diversity analysis (Nei, 1972). Only the eight RFLP loci that were shared across all populations were included in the hierarchical analysis. Genotype diversity (G) in a population, based on the comparison of either multilocus haplotypes or DNA fingerprinting patterns combined with multilocus haplotypes, was calculated using the measurement proposed by Stoddart & Taylor (1988).The genetic similarity was assayed in several ways. Individual RFLP loci were compared directly in terms of allele frequencies, number of alleles per locus, Nei´s diversity, Neis´s genetic distance and identity. If two populations were not similar in size, then the gene flow (Nm) within each geographic region and among the regions was estimated with the method described by Nei (1972) who estimated the average number of individuals that migrate between the populations per generation. If Nm < 1 local populations will differentiate; if Nm > 1 there will be little differentiation among populations. Isolates with the same DNA fingerprints and multilocus haplotype were assumed to be individual members of the same clone and were counted only once in the analysis, as was demonstrated in previous experiments (McDonald & Martínez, 1991).To compare allele frequencies in the two populations, was used only one representative of each clone to calculate the clone corrected allele frequencies.

Table 1. Reference isolates of *Septoria tritici* from Los Hornos locality, Buenos Aires Province.

Locality[a]	Date of sampling throughout vegetative stage	Isolate	Haplotype[c]	Nº of isolates with each haplotype.	Haplotype frequency%
STAR98[b]LH[a]	First sampling 05-jun	LHA3	11731611	1	1.72
		LHA30	11111211		
		LHA31	11130211	1	1.72
		LHA33	11011611	1	1.72
STAR98LH	Second sampling 24-ago	R 21	11311211	1	1.72
		R2	11111311	1	1.72
		R51	71111	1	1.72
		R26	11111318	1	
		LHB	11111211	1	1.72
		UAB3	11311211	1	1.72
		UAB5	11111211		
		UAB1	11111211		

Table 1. Continued.

		UAB2	11111211		
		UAB4	11111211		
STAR98LH	Third sampling 06-oct	LH14	11111211	1	1.72
		LH140	11011211	1	1.72
		LH15	11111211		
		LH150	11111211		
		LH151	11111211		
		LH1	11121611		
		LH11	11121611	2	3.44
		LH13	11713211	1	1.72
		LH132	11111211		
		LH132	11111211		
		LH2	11111211		
		LH224	11111211		
		LH225	11111211		
		LH226	11111211		
		LH227	11111211		
		LH228	11111211		
		LH228	11111211		
		LH241	11111211		
		LH242	11111211		
		LH16	13111211	1	1.72
		LH161	13112111	1	1.72
		LH160	11083211	1	1.72
		LH533	11111611	1	1.72
		LH551	11411611	1	1.72
		LH552	11111611	2	3.44
		LH553	1711611		
		LH41	11111211		
		LH410	11111211		
		LH411	11121211		
		LH42	11111211		
		LH420	11111211	21	36.20
		LH421	11011011	1	1.72
		LH621	21101611	1	1.72
		LH622	21001618	1	1.72
		LH623	21421618	1	1.72
		LH631	11121211	2	3.44
		LH632	11111211		
		LH633	11211211	1	1.72
		LH661	13111211		
		LH632	11111211		
		LH30	11111211	5	8.62
		LH661	13111211		
		LH662	13111211		
		LH663	13111211	4	6.89

Table 1. Continued.

[a]Los Hornos locality; [b]STAR98 *Septoria tritici* Argentina year of collection 1998. [c] [h]Each digit in the haplotype corresponds to the allele present at each of the eight RFLP loci identified by a specific probe-enzyme combination.

Table 2. Reference isolates of *Septoria tritici* from Balcarce, Buenos Aires Province.

Locality[a]	Variety of origin[c]	Code [d]	Isolate e f g	Haplotype[h]	N° of isolates with each haplotype.	Haplotype frequency%
STAR98[b] BL[a]	A	1	A1 1	11111211		
		2	A1 2	11111111	1	1.61
		3	A1 3	11111211		
		4	A2 1	13330161		
		5	A2 2	11111211		
		6	A2 3	13333611		
		7	A3 1	11111211		
		8	A3 2	23101211	1	1.61
		11	A3 3	11321611	1	1.61
		12	A4 3	11101211	1	1.61
		13	A5 1	11111211		
		14	A5 2	11010000	1	1.61
		17	A6 2	11111211		
		18	A6 3	11101111	2	3.22
STAR98BL	B	20 0	B1 1	152111		
		20 I	B1 2	11010011	2	3.22
		20II	B1 3	311		
STAR98BL	C	22	C1 2	11411211	1	1.61
		84	C3 2	21111611	1	1.61
		86	C4 1	11421211		
		87	C4 2	21411611	1	1.61
STAR98BL	D	26	D1 2	13411211	1	1.61
		27	D1 3	11611211	1	1.61
		29	D2 2	11611611	1	1.61
STAR98BL	E	31	E1 1	11111211		
		32	E1 2	11111211		
		37	E2 3	11111211		
		38	E3 1	11111211		
		40	E3 3	11111211		
		41	E4 1	11111211		
		43	E4 3	11000111	2	3.22
STAR98BL	F	45	F1 2	11433211	1	1.61
		46	F1 3	11011211		
		47	F2 1	13333611	2	3.22
		49	F2 3	11111211	9	14.50
		50	F3 1	11111611	2	3.22

Table 2. Continued.

		52	F3 3	11411611		
		54	F4 2	11521211	1	1.61
		56	F4 3	11411611		
		57	F5 1	11131611	1	1.61
		58	F5 2	13431611	1	1.61
		59	F5 3	13331611	1	1.61
STAR98BL	G	66	G2 3	13421611		
		67	G3 1	1343611		
		69	G3 3	13433611	4	6.45
STAR98BL	H	70	G4 1	11421211	2	3.22
		71	G4 2	13421611		
		76	G5 3	13421611	3	4.83
		77	G6 1	11011211	2	3.22
STAR98BL	I	89	H1 1	11811611	1	1.61
		90	H1 2	11811611	1	1.61
		92	H2 1	11111611		
		93	H2 2	11111611		
		94	H2 3	11411611		
		95	H3 1	11111211		
		99	H4 2	11113211		
STAR98BL	J	106	I1 1	11111211		
		108	I1 2	11111211	8	12.90
		109	I1 3	23121211	1	1.61
		110	I2 1	11411611	3	4.83
		111	I2 2	11111611		
		114	13.1	11111611	2	3.22

[a] Balcarce locality; [b]STAR98 *Septoria tritici* Argentina year of collection 1998.
[c]Varieties from Balcarce A= Buck Nanque, B= Klein Pegacius, C= Bonaparte Patzuco, D= Buck Arrayan, E= ProInta Oasis, F= Buck Charrua, G= ProInta Federal, H= Klein Cacique, I= Klein Cacique, [d] number of each isolate; [e] place of collection; [f] leaf from the plant; [g] pycnidium from the leaf, [h]Each digit in the haplotype corresponds to the allele present at each of the eight RFLP loci identified by a specific probe-enzyme combination.

Table 3. Information summary for two populations of *Septoria tritici* from Argentina.

	Los Hornos population	Balcarce population
Total isolates	58	62
Nº of genotypes	35	39
Nºof alleles	24	22
Isolates having fingerprint data	55	58
Nº of fingerprinting patterns	14	13
Fingerprint pattern types	A,E,F,GM,N,O,P,R,S,U,V,W,X,	A,B,D,E,H,I,K,L,M,,P,Q,R,V

Results

From of the 137 isolates coming from different areas of the Argentine wheat region, only 120 were characterized using the RFLP technique with P32 labeled probes. The pSTL70 fingerprinting probe hybridized many DNA fragments of different sizes in isolates from field populations of both places. All leaf samples were processed for isolation of the fungus, followed by fungus culture, DNA extraction, Pst1 enzyme digestion, radioactive hybridization and X ray film detection. Some of the isolates did not yield good quality DNA for the restriction enzyme digestion process. This explains the loss of 17 isolates in the samples of the populations.

In total, 24 alleles were found for Los Hornos population and 22 alleles for Balcarce population at the eight RFPL loci (Table 3). Despite the difference in the number of alleles, Nei´s measure of genetic diversity across all loci was different for both populations (0.2619 for Los Hornos and 0.3161 for Balcarce (Table 4).

Among the 58 isolates of Los Hornos and 62 of Balcarce (Table 2) with complete data from individual RFLP loci, 35 multilocus haplotypes for the first locality and 39 for the second locality, were registered. Seven new haplotypes (3a, 20a, 71a, 37a, 47a, 52a, 58a) were added to the list published on Internet (*Septoria tritici* RFLP alleles, Official allele numbers and approximate fragment sizes as July 2000).The haplotype frequency in % (times that each haplotype is repeated over the total) varied from 1.72 (1 time) to 36.20 (21 times) for Los Hornos population and 1.61(1 time) to 14.50 (9 times) for Balcarce population (Table 1 and 2). Genotype diversity was greater in the Balcarce population (G=31.61 or 26.34% of the theoretical maximum of 120) than in the Los Hornos population (G=26.19 or 21.82% of the theoretical maximum of 120). On the other hand the mean genetic diversity between populations-Nei´s formula (*Ht*= 0.405) was high for the 8 loci of RFLP (Table 5). This result implies that a significant difference exists between the populations of the two localities.

The result of the observation at a micro geographical level (ex. fungus isolated from 3 leaves of the same plant on the same place of collection) for Los Hornos (Table 1.-LHA30, LHA31, LHA33 or LH621, LH622, LH623·) and Balcarce (Table 2.-A31, A32,A33 or C41, C42) populations , demonstrated they had different genotypes of the pathogen when they were isolated from each of the three leaves; the same result was observed comparing leaves from different plants on the same place of collection (LH633, LH661 for Los Hornos or G23, G33 for Balcarce).

The isolates of Los Hornos and Balcarce populations showed a high number of fragments in the hybridization patterns with the pSTL70 probe. Fifty eight multilocus haplotypes and thirteen fingerprint patterns were registered for Los Hornos population and 55 multilocus haplotypes and fourteen fingerprint patterns for Balcarce population (Table 3). Many isolates of both populations had from one to several haplotypes for each fingerprint pattern. In Los Hornos population the E fingerprint pattern was present on 14 different haplotypes but it corresponded 3 times with the same 11211611 haplotype. In Balcarce the same fingerprint was present on 11different haplotypes, but it corresponded 8 times with the 11111211 haplotype. This last result is showing that there are clones in both populations. Some genotypes were detected as shared across the populations. In other cases several individuals in the two populations had the same multilocus haplotypes but different DNA fingerprints indicating that they were not the same clone.

The alleles frequencies (Table 4) were significantly different from the 8 loci of RFLP. Only for two RFLP locus the chi-square test was significant (P>0.005). Then the Argentine population must be compared with other continental populations -the Swiss and the United State (Oregon)- as independent populations. Estimates of Nei´s measures of gene diversity (Ht), total gene diversity (Hs) population differentiation (Gst), and gene flow (Nm) for all loci were summarized in Table 5. Over a total of 834 individuals, there was a 40% of gene diversity between native populations, and the total population differentiation was 11% showing that the differentiation between native and foreign populations exists. The low values of Gst indicate that these populations were virtually indistinguishable for these eight RFLP loci.

The average number of migrants (Nm) is 3.68. This number meant that 3 to 4 individuals would need to be exchanged across populations each generation to maintain the observed level of genetic similarity. Moreover the amount of gene flow between populations was high when all the populations were compared.

The analysis of the Genetic Identity and Genetic Distance between populations (Table 6) showed that the genetic distance was small when comparing the

Table 4. Clone- corrected allele frequencies, Nei's measures of gene diversity for eight restriction fragment length polymorphism loci in two field populations of *Septoria tritici* from Buenos Aires Province.

Probe-enzyme comb	Allele designation McDonald et al., 1995[a]	Los Hornos population (N=58)[b]	Balcarce populations (N=62)[b]	Total (N=120)[c]	X²	C	Probability(P =0.005)	Gene diversity(h) for L.Hornos population	Gene diversity[f]	Gene diversity(h) for Balcarce-population N	Gene diversity[f]
SS14	1	0.94	0.93	1.87	1.74n.s.	1	7.88	35[d]	0.1078[h]	41	0.1356
	2	0.06	0.07	0.13							
SS2	1	0.94	0.80	1.74	8.66**	1	7.88	35	0.1078	41	0.3141
	3	0.06	0.20	0.26							
SL31	1	0.19	0.26	0.45	9.74n.s.	9	23.59	36	0.7330	39	0.7850
	3	0.03	0.10	0.13							
	4	0.06	0.28	0.34							
	6	0.00	0.05	0.05							
	7	0.14	0.00	0.14							
	8	0.03	0.03	0.06							
	10	0.44	0.23	0.67							
	11	0.08	0.00	0.08							
	12	0.03	0.00	0.03							
	15	0.00	0.05	0.05							
SS43	1	0.78	0.68	1.36	5.16n.s	2	10.60	36	0.3688	40	0.4912
	2	0.14	0.15	0.29							
	3	0.08	0.18	0.26							
SL10	1	0.91	0.84	1.75	24.68***	2	10.60	35	0.1600	43	0.2791
	3	0.03	0.14	0.17							
	11	0.06	0.02	0.08							
SL53	2	0.63	0.50	1.13	4.25n.s.	3	12.84	35	0.5159	40	0.5238
	3	0.09	0.00	0.09							
	6	0.29	0.48	0.77							
	11	0.00	0.03	0.03							
SS192A	1	1.00	1.00	2.00				37	0.0000	43	0.0000
SS192B	1	0.95	1.00	1.95	7.20n.s.	1	7.88	37	0.1023	43	0.0000
	8	0.05	0.00	0.05							
MEAN								36	0.2619	41	0.3161

[a] All alleles are based on the digestion of DNA with PstI. [b] Allele frequencies for each population. [c] Total Allele frequencies. X² test is the heterogeneity of alleles frequencies among populations. C Degrees of freedom. P Probability P>0.005. [e] Sample size. [h] Gene diversity.

population of Los Hornos with the other populations, showing a high level of similarity, but the genetic distance of Los Hornos and Balcarce populations was mayor comparing with Oregon and Swiss populations. Salamati *et al*. (2000) suggested that the similarity among populations on a regional basis was explained because the gene flow was significant over spatial scales of at least several hundred kilometers. It found that genetic distances among fields within a region were small, while genetic distances among different continents were larger for the *Rhynchosporium secale* populations.

Table 5. Population structure and gene flow among Los Hornos, Balcarce, Oregon and Swiss populations.

RFLP Locus	N	Ht[c]	Hs[d]	Gst[e]	Nm[f]
STS14[a]	835[b]	0.2633	0.2398	0.0895	5,089
SS2	833	0,384	0,3604	0,0616	7,612
SL31	829	0.7758	0.6880	0.1132	3,918
SS43	832	0.5393	0.5125	0.0497	9,562
SL10	836	0.4206	0.3870	0.0798	5,765
SL53	834	0.7298	0.5425	0.2567	1,447
SS192A	836	0.0598	0.0579	0.0315	1,538
SS192B	837	0.0953	0.0895	0.0613	7,656
Average	834	0.4085	0.3597	0.1195	3,684

[a] All alleles are based on the digestion of DNA with PstI, [b]Sample size, [c] gene diversity among populations, [d] total gene diversity, [e]population differentiation, [f] gene flow.

Table 6. Table 6. Nei´s measures (in %) of Genetic Identity and Genetic Distance among *Septoria tritici* populations from Los Hornos and Balcarce (Argentina), Oregon (USA) and Switzerland.

LosHornos	Balcarce	Oregon	Swiss
Genetic Identity[a]	98.13	90.74	85.96
Genetic Distance[a]	1.80	15.00	9.72
Balcarce	LosHornos	Oregon	Swiss
Genetic Identity	98.13	90.74	85.96
Genetic Distance	1.80	18.00	11.00

[a] Nei´s measures obtained over a total of 834 isolates from four populations.

Discussion

RFLP markers used in this study revealed a high degree of genetic variability in the two Argentine populations. Higher genotype diversity was observed in the population from Balcarce than in Los Hornos. This would be likely do to the different sampling strategy used to collect the isolates from Los Hornos. The shorter distance between sampling points, choose on a hierarchical sampling, increased the likelihood of finding the same clone resulting from splash-dispersal of conidia as was demonstrated by Salamati et al. (2000). In coincidence with the same author the analysis of the local populations shown that the genetic distance among field populations were relatively small within continents and larger between continents. The complete Balcarce sample included 62 isolates from 57 leaves practically, each isolate coming from a different leaf; on the other hand, Los Hornos sample had 58 isolates coming from 40 leaves of different wheat varieties and different times of collection. Both populations shared the same alleles; different sites from the same population also repeated the same alleles. Most of the additional alleles present in the Balcarce population occurred at a low frequency, often in only one individual.

The genotypic diversity of the Balcarce population, which was 31.61% , was higher than that of the Los Hornos population, which was only 26.19% . Most of the isolates in the Los Hornos sample with the same multilocus haplotype were isolated from the same leaf. Genotypic diversity within populations and similarity over regional spatial scale was explained because regular sexual recombination was occurring rather in *Septoria tritici* than in *Rynchosporium secalis* (Salamati *et al.,* 2000), *Stagonospora nodorum* (Mc Donald *et al.,* 1994) and *Phaeosphaeria nodorum* (Keller *et al.,* 1997) populations. This was explained because the ascospores from the teleomorph were dispersed over distances of up to hundred of kilometers (Shaw & Royle, 1989; Cordo *et al.*, 1990/ 1991; Cordo *et al.*, 2005).

In the Balcarce field, each multilocus haplotype was repeated from one to twenty one times maximum; but in Los Hornos more than one multilocus haplotype was repeated several times in coincidence with the same DNA fingerprint. These haplotypes were considered clones and it existence was confirmed by the low frequency of the mating type MAT1.2 associated to that locality (Albani *et al.,* 2005). They appeared because Los Hornos are not an endemic area for the leaf blotch of wheat and the varieties that are checked for resistance are artificially inoculated with the pathogen, then the gene diversity is low in this field. In coincidence with McDonald *et al.* (1998) and Jurgens *et al.* (2006), these isolates could be individuals that were not sexually compatible to create major diversity, for climatic or other adversities, leading to genetic disequilibrium

The field populations of the fungus exhibited high degrees of gene and genotype diversity distributed on very small spatial scales. The micro geographical level observations showed a higher variation on type and number of genotypes for the Balcarce than for the Los Hornos population. In general, different genotypes were often found within a single lesion, and most lesions on the same leaf had also different genotypes. This result demonstrated, in coincidence with Boerger *et al.* (1993), that a lesion is often the result of a co infection by two or more genotypes.

The genetic distance, for native populations, were very small, considering that the geographic distances between them was 500km; the North American and European populations, separated by to 7000km, had a low increase of this genetic distance. Then the high degree of similarity could be caused by the gene flow on a regional scale and between continents (Banke *et*

al., 2004; Banke & McDonald, 2005; Boerger *et al.,* 1993; Zhan *et al.*, 2003).

The results of this contribution are in agree with Keller *et al.* (1997) who demonstrated that ascospores are the primary agent for unifying geographically separated populations on a regional scale. Added to this, Cordo *et al.* (2005) showed that ascospores were the most significant component of the *Mycosphaerella graminicola* life cycle in the wheat producing areas in Argentina. Their release was registered in the vegetative and debris wheat states for the analyzed periods. According to these experience the high degree of gene flow among populations would be associated neither the pycnidiospores presence as dominant in the life cycle of the pathogen nor the infected seeds that could act as human dispersal mechanism (Keller *et al.,* 1997). Los Hornos population resulted different because the lineages clonal of *Septoria tritici* were probably originated from the inoculations applied for the resistance tests.

If it is assumed that *Septoria tritici* did not recently colonize Argentine, the high degree of similarity could be explained from the most likely center of origin for this pathogen. Banke *et al.,* (2004) demonstrated that the New World areas (where the South Cone is considered), appeared less likely to represent ancestral populations because they had lower diversity, whereas Israel and Europe appeared to be the ancestral populations because they shown the highest genetic diversity. This pattern was related with the fact that the wheat has been grown in the Old World for thousands of years, but in the New World for only hundreds years. The movement of the fungus from Israel and into Europe could have been from wind blown ascospores or via transport on infected seed or straw. Ascospores movement produced a natural gene flow out of the possible center of origin and into European populations that could explained the finding that more haplotypes were found in European than in New World populations.

At least, another way of dispersion could be an alternate host of *S. tritici*, producing pycnidia, that constitute a continuous host population where ascospores (Boerger *et al.*, 1993; Linde *et al.,* 2002) would maintained a uniform source of inoculum that infects the wheat field each autumn This way of transmission was not demonstrated in Argentina.

Conclusions

We used DNA restriction fragment polymorphism
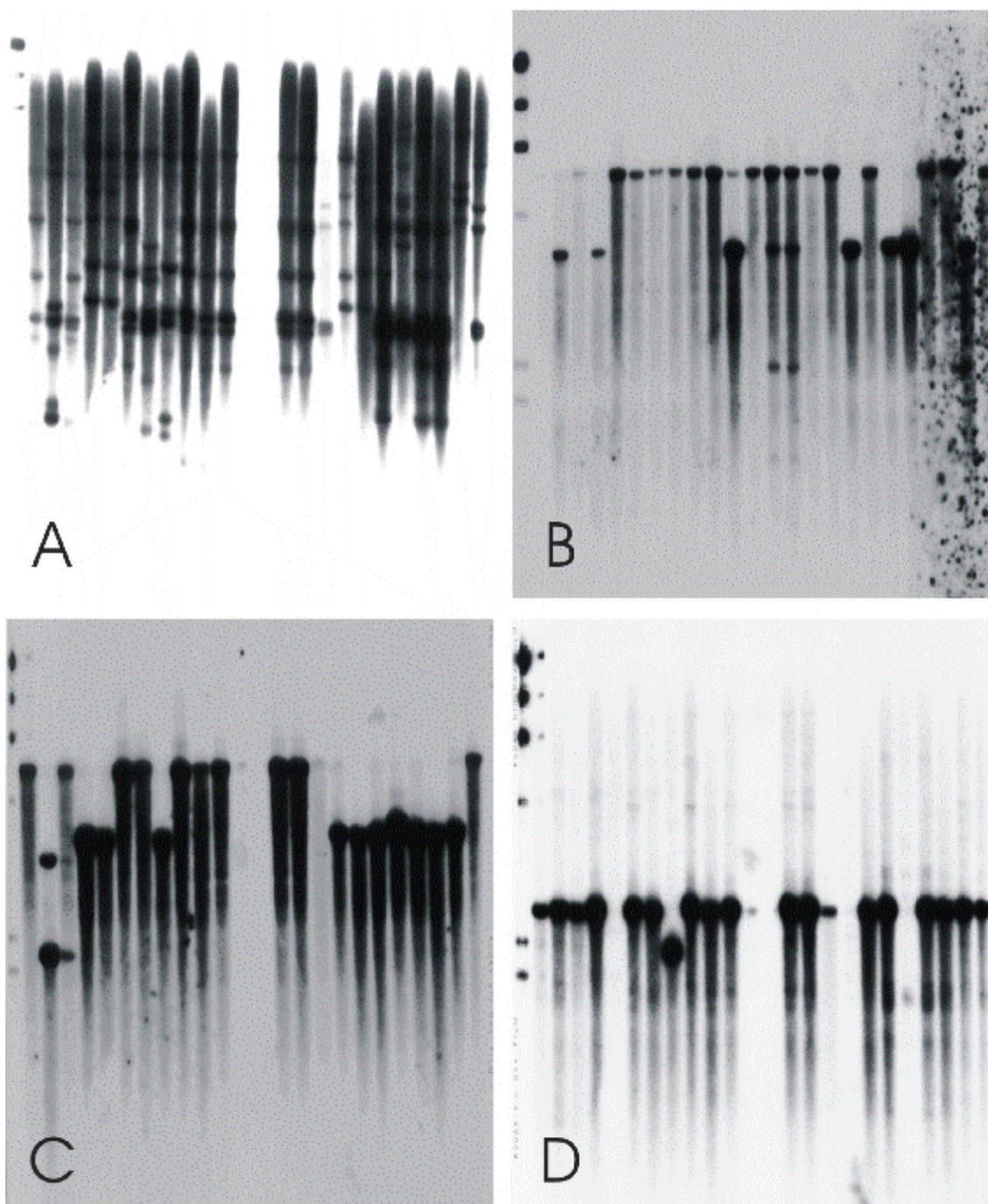
Fig.2- Examples of DNA fingerprints and RFLPs in nuclear DNA of *Septoria tritici* isolates sampled from a Balcarce field. The same isolates are shown in each panel. All DNA was digested with PstI .(A) Probe pSTL70 hybridized to a dispersed repetitive DNA family. (B) Probe pSTL53 hybridized to two loci located on different chromosomes. The upper bands represent alleles at one locus and the lower bands represent alleles at a second locus. (C) Probe pSTL10 hybridized to one locus. (D) Probe pSTS 2 hybridized to one locus.

(RFLP) markers labeled with radioactive compounds to assess the potential for gene and genetic diversity and for gene flow between geographically separated populations.

The results about the genetic compositions of two populations separated by 500 km are showing shared

haplotypes. This has significant implications for wheat breeding programs that seek to incorporate resistance to *S. tritici*. In coincidence with Boerger *et al*. (1993), our evidences for gene flow suggest that plant breeders in Argentina are driving the breeding process well. They are testing the resistance of their cultivars at many locations away from the area of local adaptation. The fine scale of patterns with genetic variability suggests that plant breeders should use a wide spectrum of pathogen genotypes when testing wheat resistant cultivars to this pathogen in any location.

Acknowledgements

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